Biochemistry

DEVELOPMENT OF AN ISOLATION AND PURIFICATION PROTOCOL FOR THE *PODOSPORA ANSERINA* AOX PROTEIN

Jeanean M. Ghering
Timothy L. Stemmler*
Department of Chemistry
Northern Michigan University
1401 Presque Isle Ave.
Marquette, MI 49855
jghering@nmu.edu

Cellular respiration is accomplished in part by the mitochondrial protein cytochrome oxidase. Cytochrome oxidase catalyzes the four-electron reduction of O_2 to H_2O which is coupled with ATP synthesis. The enzyme requires two heme groups and two copper ions to effectively perform cellular respiration. Under iron or copper deficient conditions in humans, cytochrome oxidase reactivity is decreased (or becomes non-existent) and there is a buildup of O_2 in the mitochondria. This O_2 buildup causes oxidative stress in the cell.

In the fungus *Podospora anserina*, removing cytochrome oxidase production triggers expression of an alternative oxidase (AOX) that not only increases the cells defense against mitochondrial DNA damage but also increases the life span of these cells. This alternative oxidase appears to perform O_2 metabolism in the absence of cytochrome oxidase and does so in a more efficient manner, prolonging the cells life expectancy by 10 to 1000 times their normal value (without decreasing growth rates). Homologous proteins have also been observed in plants and protozoans. Although its mechanism for respiration is unclear, *P. anserina* AOX appears to utilize a redox active iron center to catalyze the conversion of dioxygen and ubiquinol to water and ubiquinone, respectively. Based on spectroscopic data and sequence comparisons of similar well-characterized proteins, *P. anserina* AOX was proposed to contain dinuclear iron active site.

The main effort of this project was to clone and over-express *P. anserina* AOX because this would allow our lab to characterize this enzymes structure and reactivity. Our first priority was to get large concentrations of pure protein that would provide sufficient material to characterize this enzyme's structure and reaction mechanism. We have cloned and over-expressed a truncated form of this protein, using an E. coli pET-24a expression system. An existing isolation and purification protocol was then modified so that a large amount of semi-pure AOX protein was obtained.